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(12) # EUROPEAN PATENT APPLICATION

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The title of the invention has been amended (Guidelines for Examination in the EPO, A-III, 7.3).

(54) **Microbiocidal composition.**

(57) The present invention relates to a composition having microbicide effect and comprising iodine and the enzyme lactoperoxidase, whereby it comprises lactoperoxidase in an amount of at least 10 mg/l, a peroxide donor in an amount that gives at least 0.5 mM H_2O_2, I^- in a concentration of at least 10 ppm, and a pH adjusting agent in such an amount that pH is 3.25-7.0, when lactoperoxidase is used, and 3.5-6, when horse radish peroxidase is used, preferably 4.5-6.5.

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TABELL 1.

Example	1	2	3	4	5	6	7	8	9
Composition									
LP mg/l	10	10	10		20	20	10		
HP U/l				100		100		100	100
GOD U/l	100	100	100	100	100	100	100	100	100
Glucose %	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3	0,3
NaSCN ppm					40	40		6	
MgO_2 (25%) ppm									
Iodide as NaI ppm	10	100	100	100	100	100	100	100	100
Urea g/l	80	80	20	20	20	20			
Carbamide peroxide									
Citric acid g/l	0,02	0,000048							
Mono-Na-citrate g/l			4,2	4,2	4,2	4,2	2,1	2,1	2,1
Tri-Na-citrate g/l			7,8	7,8	7,8	7,8	3,9	3,9	3,9
pH initially	5,5	6			5,35	5,35	5,5	5,48	5,47
after 30 min							5,47	5,46	5,49
24 hrs		5,1			5,32	5,33	5,44	5,43	5,45
48 hrs		5			5,3	5,29	5,41	5,41	5,44
LP determ. U/l									
after 30 min		0,153			0,42	0,56	0,16	0,21	0,3
24 hrs		0,024			0,28	0,49	0,03	0,21	0,24
48 hrs					0,02	0,17	0,01		
Absorbancy 460 nm									
after 10 min	0,027	0,031			0,004	0,004	0,028	0,048	0,09
24 hrs		0,075			0,003	0,003	0,055	0,051	0,041
48 hrs		0,078			0,045	0,045	0,028	0,041	0,014

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TABELL 1.

Example	10	11	12	13	14	15	16	17	18
Composition									
LP mg/l	10	10	10	10	10	10	10	10	10
HP U7l				100				100	
GOD U/l	100		100						
Glucose %	0,3		0,3						
NaSCN ppm	6	6	6	6	6	6		6	6
MgO_2 (25%) ppm		300		300	300			300	300
Iodide as NaI ppm	100	100	500	1000	1000	100	100	100	100
Urea g/l									
Carbamide peroxide						95	95		
Citric acid g/l									
Mono-Na-citrate g/l	2,1	2,1	2,1	2,1	2,1	2,1	2,1	2,1	2,1
Tri-Na-citrate g/l	3,9	3,9	3,9	3,9	3,9	3,9	3,9	3,9	3,9
pH initially		5,9		5,44	5,44	5,48	5,46	5,43	5,44
after 30 min		5,8(60 min)		5,93	5,93	5,52	5,53	5,88	5,88
24 hrs				5,93	5,93	5,51	5,53	5,89	5,89
48 hrs									
LP determ. U/l									
after 30 min		0,011		0,3	0,3	0,33	0,37	0,58	0,29
24 hrs		0		1,1-0,1	1,1-0,1	0,001	0,002	0,08	0
48 hrs									
Absorbancy 460 nm									
after 10 min		0,24		0,877	0,895	0,222	0,25	0,207	0,221
24 hrs		0,2		0,699	0,697	0,208	0,239	0,194	0,203
48 hrs		0,19							

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TABELL 1.

Example	19	Iodophor I pH orig	Iodophor I pH 5,5	Iodophor II pH orig	Iodophor II pH 5,5	20	21
Composition							
LP mg/l	1					2.5	2.5
HP U/l							
GOD U/l	100						
Glucose %	0,1						
NaSCN ppm	6						
MgO_2 (25%) ppm							
Iodide as NaI,ppm	60	60	60	60	60	60	60
Urea g/l							
Carbamide peroxide	9,5					94	94
Citric acid g/l	0,05 mM					0.005M	0.055M
Mono-Na-citrate g/l	pH 5,5					pH 5.5	pH 3.5
Tri-Na-citrate g/l	citrate buffer					citrate buffer	citrate buffer
pH initially	5,5	2,74	5,4	4	5,5	5.07	3.64
after 30 min	5,6	2,75	5,5	3,8	5,5		
24 hrs	5,6	2,95	5,5	3,8	5,5	5.1	3.6
48 hrs						5.1	3.6
LP determ. U/l							
after 30 min	0,018						
24 hrs							
48 hrs							
Absorbancy 460 nm							
after 10 min	0,017	0,444	0,546	0,697	0,820		
24 hrs	0,00	0,404	0,516	0,677	0,791		
48 hrs	0,04						

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TABELL 2.

Example / Bacteria	1	2	3	4	5	6	7	8	9
E.coli inoc.	$9,7x10^6$	$1,7x10^6$	$1,6x10^7$	$1,6x10^7$	$6,6x10^6$	$6,6x10^6$			
30 sek	$7,0x10^1$	<1	<1	<1	$>10^6$	$>10^6$			
2 min	<1	<1	<1	<1	$>10^6$	$>10^6$			
10 min	<1	<1	<1	<1					
60 min	<1	<1	<1	<1	<1	<1			
120 min									
240 min					<1	<1			
Staph.aureus inoc.	$1,8x10^6$	$2,9x10^6$	$2,4x10^7$	$2,4x10^7$	$1,9x10^7$	$1,9x10^7$	$5,5x10^7$	$3,7x10^7$	$3,7x10^7$
30 sek	$>10^7$	$>10^7$	<1	<1	$>10^7$	$>10^7$			
2 min	$1,8x10^6$	<1	<1	<1	$>10^7$	$>10^7$			
10 min	<1	<1	<1	<1			30	<10	<10
60 min	<1	<1	<1	<1	$>10^7$	$>10^7$	<10	<10	<10
120 min							<10	<10	<10
240 min					$3,2x10^4$	$7,2x10^6$			
Strep/Staph[1] inoc.	$2,2x10^6$	$1,5x10^6$			$5,3x10^6$	$5,3x10^6$	$3,0x10^7$	$2,8x10^7$	$2,8x10^7$
30 sek	<1	10			$>10^6$	$>10^6$			
2 min	<1	90			$>10^6$	$>10^6$			
10 min	<1	<1					<10	<10	<10
60 min	<1	<1			$2x10^2$	$3x10^2$	<10	<10	<10
120 min							<10	<10	<10
240 min					<1	<1			

1) Strep. agal S-B 8 has been used for Examples 1 and 2. Strep. uberis has been used for Examples 5 and 6. Staph. aureus has been used for Examples 7-9, whereby the solutions were 20 hrs old. The solutions 1-6 were 1 hr old.

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TABELL 2.

Example	10	11	12	13	14	15	16	17	18
Bacteria									
E.coli inoc.									
30 sek									
2 min									
10 min									
60 min									
120 min									
240 min									
Staph.aureus inoc.	$5,6x10^7$	$6,2x10^8$	$5,6x10^7$	$3,8x10^7$	$3,8x10^7$	$4,9x10^7$	$4,9x10^7$	$4,5x10^7$	$4,5x10^7$
30 sek				<10	$4,4x10^2$	50	$2,9x10^2$	10	$6,5x10^2$
2 min				<10	<10	<10	40	<10	<10
10 min	70	<10		<10	<10	<10	<10	<10	<10
60 min	<10	<10	<10	<10	<10	<10	<10	<10	<10
120 min									
240 min	$2,0x10^3$	<10	<10						
S.aureus [2] inoc.	$3,8x10^7$	$7,7x10^6$	$3,8x10^7$	$3,8x10^7$	$3,8x10^7$	$4,9x10^7$	$4,9x10^7$	$3,1x10^7$	$3,1x10^7$
30 sek				$1,1x10^2$	<10	$6,0x10^2$	$2,0x10^3$	<10	20
2 min				20	<10	<10	100	<10	<10
10 min	<10	<10	<10	<10	<10	<10	<10	<10	<10
60 min	<10	<10	60	<10	<10	<10	<10	<10	<10
120 min									
240 min	<10	<10	$1,2x10^3$						

2) The solutions of the compositions used were 29 hrs old, with the exception for the solutions 13-18, which were 5 hrs old

TABELL 2.

Example	19	Iodophor I	Iodophor I	Iodophor II	Iodophor II	20	21
Bacteria		pH orig	pH 5,5	pH orig	pH 5,5		
E.coli inoc.							
30 sek							
2 min							
10 min							
60 min							
120 min							
240 min							
Staph.aureus inoc.	$3,8x10^7$	$3,6x10^7$	$3,6x10^7$	$4,8x10^7$	$4,8x10^7$	$3.9x10^7$	$3.9x10^7$
30 sek	$>10^7$	590	$2,0x10^3$	$4,9x10^4$	$1,7x10^7$	$3.6x10^2$	$1.3x10^3$
2 min	$>10^7$	<10	100	30	$1,1x10^5$	<10	<10
10 min	$>10^7$	<10	<10	<10	10	<10	<10
60 min	20	<10	200	<10	$4,0x10^3$	<10	<10
120 min							
240 min							
Staph.aureus inoc.[2)]	$4,9x10^7$	$2,8x10^7$	$2,8x10^7$	$3,1x10^7$	$3,1x10^7$		
30 sek	$>10^7$	$3,0x10^3$	$4,0x10^4$	<10	$2,7x10^4$		
2 min	$>10^7$	<10	500	<10	170		
10 min	$1,6x10^4$	<10	<10	<10	600		
60 min	$1,8x10^3$	<10	<10	<10	600		
120 min							
240 min							

[2)] The solutions of the compositions used were 24 hrs old

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As evident from Tables 1 and 2 a considerable microbicide effect is obtained by means of the compositions according to the present invention. The remarkable thing is that they functions well at a slightly acidic, almost neutral pH, at which pH iodophores do not function, when pH has been adjusted to substantially neutral value. In the latter case the microbicide effect has almost completely failed.

The present composition is present in a water free form, at least concerning the hydrogen peroxide donating/formating part. Thus the composition according to the invention is present preferably in dry form, but can also be present in the form of a paste which comprises two parts which are brought together at the use. Even a liquid composition can be used considering the basic demand, viz that the hydrogen peroxide donating/formating part is kept out of contact with water until in use. Besides a pure dry pulverulent mixture, the composition can be present in the form of tablets and granules as well as double layer tablets which are dissolved in a suitable amount of water prior to use.

The following Examples 22-31 were prepared for comparative testing. Thus compositions according to the present invention were prepared, as well as according to EP-A1-0 175 801. Example 24 is prepared in accordance with Example 5 of EP-A1-0 175 801, and Example 25 is the same as Ex. 24 but for a weaker buffer, 0.01M. Example 27 is the same as Ex. 24 except for the concentration of the buffer and the pH.

Table 3 below gives the different compositions of Examples 22-31.

Table 4 below gives the absorbancy at 460 nm for each of the solutions of Ex. 22-31.

Following the UV absorbancy test four compositions were picked out for a bactericide test, viz. the compositions of Ex. 22, 24, 26, and 27, respectively, whereby the compositions were tested against Staph. aureus MJ 13151/84, Staph. aureus 1243/87, Pseudomonas aeruginosa Ps. q. 41, and E. coli A126, respectively, with regard to their bactericide effect. The Staph. aureus strains were inocculated in an amount of 8×10^7 cfu/ml, Ps aeruginosa in an amount of 1.1×10^8 cfu/ml, and E. coli in an amount of 6.8×10^7 cfu/ml. Surviving cfu were tested for after 30 s, 60 s, 2 min, and 10 min, respectively. The bactericide effect obtained is shown in Table 5 below, whereby the killing effect is given in percentage of inhibition in a log scale. Zero value indicates no or very weak killing capacity, while 100% corresponds to total killing.

Table 3.

Components	22	23	24	25	26	27	28	29	30	31
LP	10	10			10		10	10		
HP			10	10		10			10	10
Phosphate buffer	0.1	0.01	0.1	0.01			0.1	0.01	0.1	0.01
Citrate buffer					0.01	0.01				
NaI, mM	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
H_2O_2, mM	0.1	0.1	0.1	0.1	0.1	0.1				
Urea peroxide							0.1	0.1	0.1	0.1
pH	7.0	7.0	7.0	7.0	5.0	5.0	7.0	7.0	7.0	7.0

Table 4.

Example	Absorbancy at 460 nm				pH	
	initially	10 min	30 min	24 hrs	initially	30 min
22	0.065	0.063	0.056	0.018	7.0	7.0
23	0.104	0.100	0.091	0.035	7.1	7.1
24	0.000	0.000	0.002	0.000	7.0	7.1
25	0.000	0.000	0.001	0.000	7.1	7.1
26	0.171	0.163	0.160	0.078	5.1	5.1
27	0.183	0.175	0.167	0.070	5.1	5.1
28	0.052	0.048	0.047	0.011	7.0	7.0
29	0.065	0.061	0.059	0.022	7.1	7.1
30	0.003	0.007	0.008	0.003	7.0	7.0
31	0.004	0.007	0.014	0.005	7.0	7.0

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 88 85 0240

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
X	US-A-4 282 324 (S.L. NEIDLEMAN et al.) * Column 2, line 38 - column 3, line 9; column 3, lines 25-32; examples; claims * ---	1-8	A 01 N 63/00 A 61 L 2/18 // (A 01 N 63/00 A 01 N 59:12 A 01 N 59:00)
X	CHEMICAL ABSTRACTS, vol. 68, no. 7, 12th February 1968, page 2665, no. 27662h, Columbus, Ohio, US; J.K. SEYMOUR: "Iodination of bacteria: a bactericidal mechanism", & J. EXP. MED. 126(6), 1063-78(1967) * Abstract * ---	1-8	
A	CHEMICAL ABSTRACTS, vol. 99, no. 23, 5th December 1983, page 458, no. 191505v, Columbus, Ohio, US; A.M. SUGAR et al.: "Susceptibility of Blastomyces dermatitidis strains to products of oxidative metabolism", & INFECT. IMMUN. 1983, 41(3), 908-12 * Abstract * ---	1-8	
A	CHEMICAL ABSTRACTS, vol. 89, no. 11, 11th September 1978, page 86, no. 85242r, 85243s, Columbus, Ohio, US; E.L. THOMAS et al.: "Cofactor role of iodide in peroxidase antimicrobial action against Escherichia coli", & ANTIMICROB. AGENTS CHEMOTHER. 1978, 13(6), 1000-5; "Oxidation of Escherichia coli sulfhydryl components by the peroxidase-hydrogen peroxide-iodide antimicrobial system", & ANTIMICROB. AGENTS CHEMOTHER. 1978, 13(6), 1106-10 * Abstracts * --- -/-	1-8	

TECHNICAL FIELDS SEARCHED (Int. Cl.4)

A 01 N
A 61 L

The present search report has been drawn up for all claims

Place of search	Date of completion of the search	Examiner
THE HAGUE	27-10-1988	FLETCHER A.S.

CATEGORY OF CITED DOCUMENTS

X : particularly relevant if taken alone
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A : technological background
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EUROPEAN SEARCH REPORT

Application Number

EP 88 85 0240

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.4)
D,A	EP-A-0 175 801 (J. KESSLER et al.) * Claims *	1-8	
			TECHNICAL FIELDS SEARCHED (Int. Cl.4)

The present search report has been drawn up for all claims

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